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NOTE

A New Non-Destructive DNA Extraction and Specimen Clearing Technique for Aphids (Hemiptera)

Slide mounting soft bodied insects such as aphids is a time-consuming task that is often regarded as art and alchemy more than science. Many techniques have been developed to prepare specimens (Essig 1948, Wilkey 1962, Martin 1983). The typical use of potassium hydroxide (KOH) or sodium hydroxide (NaOH) complicates the process as one tries to strike a balance between adequate clearing and rendering the specimen too fragile to handle. A technique developed by Hille Ris Lambers (1950) employs chloral phenol in an effort to mitigate the risks to the specimen when using KOH. However, one key ingredient of chloral phenol, chloral hydrate, is a controlled substance and is dangerous and unpleasant to use. I modified a standard DNA extraction technique that not only clears aphid specimens easily and with few complications, but also extracts the aphid's DNA non-destructively, allowing for specimen vouchering of genetic sources.

Specimens of some taxa must be destroyed in order to extract their DNA. In fact, some organisms are known only by their DNA sequences (Pace 1997). However, there are benefits in preserving the actual specimen from which the DNA is extracted. Various techniques have been developed to extract DNA from insect specimens relatively nondestructively (Phillips and Simon 1995, Johnson et al. 2001, Starks and Peters 2002). Keeping a voucher specimen from which the DNA is taken is especially useful in the case of solitary aphids, when multiple clonal individuals from a single colony are not available.

I adapted the DNA extraction protocol from Favret and Voegtlin (2004) to preserve the aphid cuticle intact for mounting to a microscope slide. The extraction solution consists of 500 µl STE buffer (pH 7.5), 25 µl of 10 mg/ml proteinase K, and 75 µl of 10% SDS (Hillis et al. 1996: 342–343). An incision along the length of the ventral aspect of the aphid abdomen is made under a microscope with a bent minuten pin mounted on the end of a thin dowel. The incision allows entry of the clearing solution into the aphid body. The entire aphid is placed in a microvial containing the extraction solution and the vial placed in a 55°C water bath overnight. The next day the aphid is removed from the buffer using sterilized forceps. DNA purification continues as per the normal protocol (Hillis et al. 1996, Favret and Voegtlin 2004), and the cuticle is dehydrated and mounted to a microscope slide. The following steps are necessary to prepare the specimen for slide mounting in Canada balsam: 10 minutes in 70% ethanol, 10 minutes in 95% ethanol, 2 minutes in glacial acetic acid, 10 minutes in a 1:1 mixture of glacial acetic acid and terpineol, and 10 minutes in pure terpineol. After the specimen is positioned on the slide in balsam, a cover slip is applied and the slide is cured on a slide warmer or in drying oven at 50°C for a week or more.

For DNA extraction, each microvial must contain only one specimen, but several can be cleared simultaneously in a single microvial if the DNA is not to be saved. The extraction solution clears the aphid similarly to conventional clearing techniques, yet leaves the aphid cuticle supple and strong. On rare occasions, large or heavily pigmented aphids need additional clearing; for these, I remove the embryos from the body and repeat the clearing cycle. Aphids cleared in DNA extraction buffer and mounted on slides are indistinguishable from those prepared by other means such

as the Hille Ris Lambers (1950) method. This technique should be adaptable to most DNA extraction protocols such as the Qiagen kit used by Johnson et al. (2001). They left lice specimens in the extraction buffer for 56 h, but I found overnight to be sufficient for most aphids, and most specimens yielded large, visible DNA pellets.

The clearing technique presented here is not only easy to use and forgiving, but also allows for non-destructive DNA extraction and specimen vouchering. This latter advantage has positive ramifications for insect taxonomy, and has allowed me to confirm the identity of *Cinara atlantica* (Wilson) in Brazil, which is not morphologically distinguishable from *C. ponderosae* (Williams): CO-1 DNA sequence from a Brazilian specimen was identical to that of *C. atlantica* from Florida (GenBank accession number AY300225), differed by one base from that of *C. atlantica* from South Carolina (AY300198), and differed from *C. ponderosae* by 15 bases (AY300194). Likewise, I also have identified a *Cinara* nymph on an imported host, *Pinus nigra* Arnold in Nebraska. Nymphs are difficult or impossible to identify morphologically and host-based keys are for European *Cinara* species only (Blackman and Eastop 1994). However, CO-1 DNA sequence data (AY300229) matched those found previously for *C. arizonica* (Wilson) (AY300222 and AY300230). This is the first record of *C. arizonica* from *P. nigra* and also the first record of this aphid species in Nebraska. Both the Brazilian and Nebraskan aphids whose DNA was extracted using the protocol described above are intact and deposited in the insect collection at the Illinois Natural History Survey, Champaign, IL: catalog numbers 18,292 and 16,756 in the insect collection database at http://www.inhs.uiuc.edu.

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